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Determination of Bioactive Peptide Molecular Mass Using Electrospray and Matrix Assisted Laser Desorption Ionization Mass Spectrometry

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ABSTRACT

Twelve bioactive peptides, ranging in molecular masses from 600 and 4500 u, were selected for ESI-MS and MALDI-TOF-MS analysis in order to assess the spectrometric data that could be accessed for rapid chemical/biological warfare agent screening purposes. Monoisotopic molecular mass data were obtained for all the peptides by ESI-MS with a tri-focusing magnetic sector instrument in the continuum mode using resolutions between 3000 and 6000 (10% valley definition). MALDI-TOF-MS data were collected with an instrument that featured a four-plate source design, pulsed ion extraction for time-lag focusing, and a 1-m linear flight tube. High resolution mass measurements with the tri-focusing magnetic sector instrument were the most accurate. Errors between theoretical and calculated monoisotopic (or average) molecular mass were in the 0.3 to 18 ppm and 10 to 96 ppm range during ESI-MS and MALDI-TOF-MS analysis, respectively. ESI-MS data may be acquired with as little as 1 to 10 pmoles of peptide at resolutions of 2000 to 3000 (10% valley definition) with the tri-focussing magnetic sector instrument. The MALDI-TOF-MS instrument described offered improved sensitivities with only 0.1 to 1 pmole of peptide being required. In both cases analyses times were rapid, with analyses taking 2 to 5 minutes per sample. Both ionization techniques, ESI and MALDI, would be suitable for rapid molecular mass screening purposes.

Executive Summary ii

Title: P.A. D'Agostino, J.R. Hancock, J.A. Tornes, Y. Dai and L. Li, "Determination of Bioactive Peptide Molecular Mass using Electrospray and Matrix Assisted Laser Desorption Ionization Mass Spectrometry", Suffield Memorandum No. 1497, 1998, UNCLASSIFIED.

Introduction: The Canadian Forces (CF) may be called on to perform peacekeeping or battlefield operations in regions of the world where there is a significant threat of chemical/biological warfare agent use. To operate effectively in these theatres the CF must be able to identify the exact nature of the chemical/biological (CB) warfare agent(s). Mass spectrometry (MS), is a powerful analytical technique for the identification of both known and unknown compounds and DRE Suffield is currently investigating this instrumental technique in fulfilment of CF agent detection and identification requirements.

Results: Twelve bioactive peptides, ranging in molecular masses from 600 and 4500 u, were selected for ESI-MS and MALDI-TOF-MS analysis in order to assess the spectrometric data that could be accessed for rapid CB screening purposes. Monoisotopic molecular mass data were obtained for all the peptides by ESI-MS with a tri-focusing magnetic sector instrument using resolutions between 3000 and 6000 (10% valley definition). During analysis of the neuropeptide Y series the instrument was operated at 6000 resolution (10% valley definition) in order to completely resolve the $(M+6H)^{6+}$ isotopic clusters used for monoisotopic molecular mass determinations. ESI-MS data were most accurate with errors between theoretical and calculated monoisotopic molecular mass being in the 0.3 to 18 ppm range. MALDI-TOF-MS data were collected with an instrument that featured a four-plate source design, pulsed ion extraction for time-lag focusing, and a 1-m linear flight tube. MALDI-TOF-MS errors between theoretical and calculated monoisotopic or average molecular mass were in the 10 to 96 ppm range.

ESI-MS data may be acquired with 1 to 10 pmoles of peptide at resolutions of 2000 to 3000 (10% valley definition) with the tri-focussing magnetic sector instrument.The MALDI-TOF-MS instrument described offered improved sensitivities with only 0.1 to 1 pmole of peptide being required. In both cases analyses times were rapid, taking 2 to 5 minutes per sample.

Significance of Results: The CF may be deployed in regions of the world where there is a significant threat of chemical/biological warfare agent use. Identification of the agent is of importance since the results of such analyses would contribute to the development of strategic and political positions regarding future Canadian military operations and would facilitate the dissemination of technical advice to in-theatre field commanders and medical personnel.

Future Goals: The CB threat spectrum includes chemical and biological warfare agents and toxins of biological origin in the "mid-spectrum" between these agents. The identification research effort has been focused on the detection and identification of these toxins of biological origin. Use of these warfare agents could easily go unconfirmed, as analytical methods have not been fully developed for their identification. DRE Suffield is now actively addressing this deficiency through the application and development of MS methods for the identification of these agents.

TABLE OF CONTENTS

INTRODUCTION

Canadian Forces (CF) may be called on to perform peacekeeping or battlefield operations in regions of the world where there is a significant threat of chemical/biological (CB) warfare agent use. To operate effectively in these theaters the CF must be able to detect and identify the CB agent(s) as rapidly as possible. The Defence Research Establishment Suffield (DRES) has been tasked to develop a highly sensitive system for the detection and identification of agents of biological origin, including peptides and proteins, that could be used as biological warfare agents. DRES has adopted a multi-disciplinary approach that includes instrumental analytical techniques, immunological methods and other technologies in order to identify as wide a variety of CB agents as possible.

The application of mass spectrometry to the analysis of biomolecules, has undergone tremendous growth due to the development of two ionization techniques, electrospray (ESI) (1,2) and matrix-assisted laser desorption ionization (MALDI) (3,4). Mass spectrometry combines exceptional sensitivity, specificity and speed for the analysis of peptides and proteins, making it a valuable analytical tool in many biochemical or pharmaceutical laboratories. It forms a cornerstone of the DRES agent detection/identification research effort and will be used for the identification of previously characterized bioactive peptides and for broad spectrum detection and identification of novel unknown toxic peptide and proteins. The DRES ESI-MS effort is currently being conducted with a tri-focusing magnetic sector mass spectrometer, with the major advantage of this instrumentation being its high resolution capability. Increasing resolution generally results in improved monoisotopic molecular and product ion mass accuracy, as well as assignment of charge state for resolved isotopic clusters.

A number of researchers have made use of the resolution of di- or tri-focusing magnetic sector instruments for molecular mass determination following electrospray ionization. Larsen and

McEwen (5) employed resolutions of 5000 and 10000 (10% valley definition) for molecular mass determination and found that errors seldom exceeded 25 ppm for several peptides. Calibration was done internally and the isotopic cluster for the +5 charge state of insulin was resolved. High resolution separation of a $(M+9H)^{9+}$ isotopic cluster was demonstrated by Cody, Tamura and Musselman for lysozyme at a resolution of 10,000 (10% valley definition). Errors associated with these measurements were in the 5 to 20 ppm range when internal calibration was employed (6). Higher errors, typically in the 5 to 90 ppm range, were observed when external calibration was used over a 10 hour period. Similar mass accuracy was achieved during the analysis of a series of thirty-seven unknown synthetic peptides, used in research studies involving synthetic vaccines, antibacterial peptides or the de novo design of helical peptides and proteins (7). All data were obtained with external calibration over a wide mass range during magnetic scanning. Errors between observed and theoretical monoisotopic molecular masses were typically in the 5 to 60 ppm range for the unknowns at sector resolutions between 2500 and 9000 (10% valley definition). Isotopic clusters for charge states up to +10 were resolved through the use of high resolution.

High resolution LC-ESI-MS data for a number of bioactive peptides were recently acquired over a wide mass range by scanning the magnetic sector and calibrating externally with polyethylene glycol standards (8). Multiply charged ions were observed and errors between observed and theoretical monoisotopic molecular masses were typically in the 5 to 30 ppm range for resolutions between 2500 and 6000 (10% valley definition). Mass accuracy was evaluated during an NATO international round robin analytical exercise where the molecular masses of five unknown peptides were to be determined. Isotopic clusters for charge states of up to +6 were fully resolved, facilitating the rapid and unambiguous assignment of charge states and calculation of monoisotopic molecular masses. Errors between theoretical and observed monoisotopic molecular masses were in the 2 to 18 ppm range (8).

Until recently, most MALDI-MS analyses with time-of-flight (TOF) instrumentation were

made under relatively low resolution conditions, resulting in the acquisition of average molecular mass data. In the past two years, MALDI TOF instrumentation has advanced significantly, largely due to the development and implementation of time-lag focusing or delayed extraction for MALDI. The time-lag focusing concept was initially developed by Wiley and McLaren in 1955 (9) and has since been applied to several different ion sources (10,11) including MALDI (12). However, significantly improved analytical performance for MALDI in terms of mass resolution (13-16) and mass accuracy (14) was shown only recently. A linear, 1-m flight path TOF instrument equipped with time-lag focusing can provide sufficiently high resolving power to resolve carbon isotope peaks for molecules with molecular weights up to 3000 (14,16). For higher masses, up to at least 30,000 u for proteins, resolution between 1000 and 1500 fwhm (full width at half maximum) is readily obtained (16,17). Combining time-lag focusing with a reflectron or ion mirror for further energy correction, MALDI TOF can achieve unit mass resolution for ions with masses up to about 10,000 u (16). At even higher masses, the resolving power of a reflectron time-lag focusing system is similar to that of a linear time-lag focusing instrument.

A comparison of ESI-MS and MALDI-TOF-MS for bioactive peptide identification, was initiated between the University of Alberta and DRES. A dozen typical bioactive peptides, ranging in molecular mass from 600 to 4500 u, were analysed using both techniques in order to assess the spectrometric data that could be accessed for rapid CB screening purposes. The isotopic clusters of lower molecular mass bioactive peptides could be resolved and the monoisotopic molecular masses were calculated using data obtained with both types of instrumentation. Monoisotopic molecular masses for the larger bioactive peptides could only be accessed with the ESI-MS instrument. Comparable average molecular mass data were generated during MALDI-TOF-MS analysis.

EXPERIMENTAL

ESI-MS Analysis

All the bioactive peptides were purchased from Sigma Chemical Company (St. Louis, MO, USA) and dissolved at 0.1 to 0.2 mg/mL in distilled water. Distilled-in-glass water was filtered through a 0.45 μm Millipore filter prior to use in the mobile phase or for diluting the peptide samples. Acetonitrile was Burdick and Jackson UV grade (Muskegon, MI, USA).

All electrospray mass spectra were acquired using a Micromass Autospec-Q tri-focusing magnetic sector mass spectrometer (Manchester, UK) equipped with the Mark II electrospray interface. The electrospray needle was operated at 7.6 kV and ions were accelerated into the mass spectrometer at 4 kV. A sampling cone voltages of 50 V was used. Nitrogen (Very Dry, Liquid Carbonic Inc., Scarborough, Ont., Canada) bath gas was introduced into the interface (80°C) at a flow rate of 300 to 400 L/hr. Nitrogen nebulizer gas was introduced at a flow rate of 14 L/hr. The electrospray interface was pumped with both a rotary and a turbomolecular pump, which enabled maintenance of $4x10^{-4}$ and $7x10^{-6}$ Pa within the source and analyser regions, respectively. Loop injections (10 μL) of the bioactive peptides were performed with an Applied Biosystems Model 140B dual syringe pump (Foster City, CA) using acetonitrile/water (50/50) at 10 μL/min.

This resulted in the acquisition of five to ten scans for each sample component during ESI-MS analysis. Resolutions of 3000 to 6000 (10% valley definition) were employed during magnetic sector scanning to facilitate accurate mass measurement of the ions formed during ESI-MS analyses. External calibrations were performed with solutions of polyethylene glycol in distilled water. Monoisotopic molecular masses for the bioactive peptides were calculated (n=5) from the most intense $(M+nH)^{n+}$ isotopic cluster for each bioactive peptide.

MALDI-TOF-MS Analysis

MALDI mass spectra were collected on a linear time-lag focusing MALDI TOF mass spectrometer. The basic construction of the instrument has been described elsewhere (14). It features a four-plate source design, pulsed ion extraction for time-lag focusing, and a 1-m linear flight tube. The ions are generated using the 337-nm laser beam from a nitrogen laser, having a pulse width of 3 ns (model VSL 337ND, Laser Sciences Inc., Newton, MA). A microchannel plate detector was used for ion detection, and a Hewlett-Packard MALDI data system was used for mass spectral recording and data processing. Mass calibrations were performed externally, using peptide and protein standards. In general, mass spectra from 50 to 100 laser shots were summed to produce the final spectrum.

The bioactive peptides were dissolved in water to generate stock solutions. Samples were prepared using a two-layer method (18). To prepare the first layer, sinapinic acid was dissolved in 60% mehanol/acetone (v/v) at a concentration of 5 mg/mL. To prepare the second layer, sinapinic acid was saturated (~15 mg/mL) in 50% acetonitrile/water (v/v) and mixed 1:1 (v/v) with the peptide. The stainless steel MALDI probe was successively polished with aluminum oxide particles to a final particle size of 0.3 μm. Onto this surface, 1.0 μL of the first-layer solution was added and allowed to dry. To the first layer, 0.5 μL of the second-layer solution was added and allowed to dry at room temperature. The total amount of peptide loaded to the MALDI probe was typically 1 pmol.

RESULTS AND DISCUSSION

Twelve bioactive peptides, ranging in molecular masses from 600 and 4500 u, were selected for ESI-MS and MALDI-TOF-MS analysis in order to assess the spectrometric data that could be accessed for rapid CB screening purposes. Each peptide was dissolved and analysed individually five times to determine either monoisotopic or average molecular mass. Monoisotopic molecular mass data (Table I) were obtained for all the bioactive peptides by ESI-MS using external calibration and resolutions between 3000 and 6000 (10% valley definition) to facilitate the complete separation of isotopic clusters. ESI-MS data were acquired in the continuum mode by scanning the magnet over a narrow mass range (approximately 150 u) such that each scan took about four to six seconds. Errors between theoretical and calculated monoisotopic molecular mass were in the 0.3 to 18 ppm range, the same range as reported in the NATO international round robin analytical exercise (8).

The MALDI-TOF-MS instrument used for the bioactive peptide analyses featured a four-plate source design, pulsed ion extraction for time-lag focusing, and a 1-m linear flight tube. Isotopic clusters for all but the higher mass neuropeptide Y series were resolved, enabling the determination of monoisotopic molecular mass for nine of the peptides. Average molecular masses were determined for the neuropeptide Y series. Table II lists the results obtained by MALDI-TOF-MS. Errors between theoretical and calculated monoisotopic or average molecular mass were in the 10 to 96 ppm range using external calibration. This compares favorably with the best of the low resolution quadrupole data reported during the NATO international round robin analytical exercise (8).

Figures 1, 2 and 3 illustrate typical mass spectra data obtained for peptides in the 1000, 2000 and 4000 u range, respectively, using both techniques. Substance P (4-11), a peptide with a monoisotopic molecular mass of 965.4793 u, exhibited a significant singly charged isotopic cluster using both techniques (Figure 1). The isotopic cluster was well resolved, enabling rapid

determination of charge state and monoisotopic molecular mass by either ESI-MS or MALDI-TOF-MS.

The presence of isotopic clusters of higher charge generally increases with mass during ESI-MS, provided there are sufficient sites available for protonation. In these cases the most intense isotopic cluster was first determined by scanning over a wider mass range (e.g., 1400 to 400 u). The mass range was then narrowed to about 150 u for monoisotopic molecular mass determination. Monoisotopic molecular masses for all the peptides in the 1000 to 2000 u range were determined from the $(M+2H)^{2+}$ isotopic cluster during ESI-MS analysis. A resolving power of 3000 (10% valley definition) enabled complete resolution of the $(M+2H)^{2+}$ isotopic clusters. Figure 2 illustrates the ESI-MS and MALDI-TOF-MS data obtained for the highest mass peptide in this mass range, bombesin. Multiple charging is less significant during MALDI experiments, particularly at lower mass, and monoisotopic molecular mass was determined from the resolved $(M+H)^{+}$ isotopic cluster.

MALDI-TOF resolution with the described configuration is typically in the 2000 to 5000 range (based on fwhm) for masses up to 3000 u. This typically decreases to the 800 to 1500 range (based on fwhm) for larger peptides and proteins with masses up to 30,000 u. The molecular mass of the neuropeptide Y series (4000 to 4500 u) were determined as average molecular masses since the isotopic clusters were not resolved by MALDI-TOF-MS. The tri-focussing magnetic sector instrument has been used successfully for monoisotopic mass measurements in the 7000 to 8000 u range using a resolving power of 9000 to 10,000 (10% valley definition). Above this mass it becomes difficult to determine which ion contains only ^{12}C content due to low relative ion intensity (7). During analysis of the neuropeptide Y series the instrument was operated at 6000 resolution (10% valley definition) in order to completely resolve the $(M+6H)^{6+}$ isotopic clusters used for monoisotopic molecular mass determinations. Figure 3 illustrates MALDI-TOF-MS and ESI-MS data obtained for neuropeptide Y (porcine), a bioactive peptide with a monoisotopic and average molecular masses of 4251.1249 and 4253.7034 u, respectively.

Databases of known peptides and proteins such as SWISSPROT can be interrogated with commercial mass spectrometry software, such as MassLynx (Micromass, UK). Searches are generally conducted on generated molecular mass data for intact peptides or their enzymatic fragments. Improved mass measurement accuracy, made possible through higher resolution during ESI-MS and pulsed ion extraction for time-lag focusing during MALDI-TOF-MS would aid in the searching process. It would reduce the number of possible peptide or peptide fragment database matches during molecular mass searching (19,20).

ESI-MS data may be acquired with as little as 1 to 10 pmoles of peptide at resolutions of 2000 to 3000 (10% valley definition) with the tri-focussing magnetic sector instrument. The benefits of increasing resolution however come at the expense of sensitivity, with about an order of magnitude more analyte being required when working at resolutions in the 6000 to 10,000 range (10% valley definition).The MALDI-TOF-MS instrument described offered improved sensitivities with only 0.1 to 1 pmole of peptide being required.

Analysis times for ESI-MS using loop injections were comparable with those noted during MALDI-TOF-MS analysis. Analysis times of 2 to 5 minutes per sample were typical. In the case of the tri-focussing magnetic sector instrument a longer "one time" setup time of 10 to 20 minutes prior to a set of analyses would be required to achieve resolution above 3000 (10% valley definition). Both ionization techniques, ESI and MALDI, would be suitable for rapid molecular mass screening purposes.

CONCLUSIONS

Twelve bioactive peptides, ranging in molecular masses from 600 and 4500 u, were selected for ESI-MS and MALDI-TOF-MS analysis in order to assess the spectrometric data that could be accessed for rapid CB screening purposes. Monoisotopic molecular mass data were obtained for all the peptides by ESI-MS with a tri-focusing magnetic sector instrument in the continuum mode using resolutions between 3000 and 6000 (10% valley definition). During analysis of the neuropeptide Y series the instrument was operated at 6000 resolution (10% valley definition) in order to completely resolve the $(M+6H)^{6+}$ isotopic clusters used for monoisotopic molecular mass determinations. High resolution mass measurements with the tri-focusing magnetic sector instrument were the most accurate. ESI-MS errors between theoretical and calculated monoisotopic molecular mass were in the 0.3 to 18 ppm range. MALDI-TOF-MS data were collected with an instrument that featured a four-plate source design, pulsed ion extraction for time-lag focusing, and a 1-m linear flight tube. Isotopic clusters for all but the higher mass neuropeptide Y series were resolved, enabling the determination of monoisotopic molecular mass for nine of the peptides. MALDI-TOF-MS errors between theoretical and calculated monoisotopic or average molecular mass were in the 10 to 96 ppm range.

ESI-MS data may be acquired with 1 to 10 pmoles of peptide at resolutions of 2000 to 3000 (10% valley definition) with the tri-focussing magnetic sector instrument. The MALDI-TOF-MS instrument described offered improved sensitivities with only 0.1 to 1 pmole of peptide being required. In both cases analyses times were rapid, taking 2 to 5 minutes per sample.

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Table I: ESI-MS monoisotopic molecular mass data obtained with a magnetic sector mass spectrometer (magnetic scanning, external calibration, 3-6000 resolution). The average error for monoisotopic molecular mass measurement was 8.5 ± 6.1ppm.

Bioactive Peptide	Theoretical Monoisotopic Molecular Mass	Calculated Monoisotopic Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Substance P (7-11)	612.3094	612.3106 612.3084 612.3107 612.3099 612.3107	612.310 ± 0.001	1.0
Substance P (6-11)	740.368	740.3747 740.3756 740.3755 740.3754 740.3756	740.375 ± 0.001	9.5
Substance P (4-11)	965.4793	965.4796 965.4764 965.4796 965.4787 965.4798	965.479 ± 0.001	0.3

Bioactive Peptide	Theoretical Monoisotopic Molecular Mass	Calculated Monoisotopic Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Substance P	1346.7281	1346.7432 1346.7448 1346.7444 1346.7450 1346.7414	1346.744 ± 0.001	12
Bradykinin	1059.5613	1059.5620 1059.5616 1059.5596 1059.5570 1059.5582	1059.560 ± 0.002	1.2
Lys-Bradykinin	1187.6563	1187.6648 1187.6632 1187.6624 1187.6682 1187.6654	1187.665 ± 0.002	7.3
Ile-Ser-Bradykinin	1259.6775	1259.6732 1259.6784 1259.6888 1259.6874 1259.6862	1259.683 ± 0.007	4.4

Bioactive Peptide	Theoretical Monoisotopic Molecular Mass	Calculated Monoisotopic Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Met-Lys-Bradykinin	1318.6968	1318.6728 1318.6712 1318.6800 1318.6852 1318.6820	1318.678 ± 0.006	14
Bombesin	1618.815	1618.7952 1618.8030 1618.8072 1618.8142 1618.8116	1618.806 ± 0.008	5.6
Neuropeptide Y (sheep)	4237.1093	4237.1525 4237.1831 4237.1813 4237.1693 4237.1771	4237.17 ± 0.01	14
Neuropeptide Y (porcine)	4251.1249	4251.1973 4251.1967 4251.1979 4251.1859 4251.1619	4251.19 ± 0.02	15

Bioactive Peptide	Theoretical Monoisotopic Molecular Mass	Calculated Monoisotopic Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Neuropeptide Y (human)	4269.0814	4269.1667 4269.1769 4269.1487 4269.1763 4269.1451	4269.16 ± 0.02	18

Table II: MALDI-MS monoisotopic and average molecular mass data obtained with a time-of flight instrument. The average error for molecular mass measurement was 32 ± 24 ppm.

Bioactive Peptide	Theoretical Monoisotopic Molecular Mass	Calculated Monoisotopic Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Substance P (7-11)	612.3094	612.32 612.26 612.28 612.27 612.27	612.28 ± 0.02	48
Substance P (6-11)	740.368	740.30 740.34 740.33 740.33 740.42	740.34 ± 0.05	38
Substance P (4-11)	965.4793	965.50 965.49 965.42 965.42 965.50	965.47 ± 0.04	10
Substance P	1346.7281	1346.81 1346.81 1346.79 1346.77 1346.60	1346.76 ± 0.09	24
Bradykinin	1059.5613	1059.44 1059.49 1059.43 1059.53 1059.43	1059.46 ± 0.04	96

Bioactive Peptide	Theoretical Monoisotopic Molecular Mass	Calculated Monoisotopic Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Lys-Bradykinin	1187.6563	1187.64 1187.63 1187.55 1187.66 1187.55	1187.61 ± 0.05	39
Ile-Ser-Bradykinin	1259.6775	1259.66 1259.66 1259.66 1259.67 1259.67	1259.66 ± 0.01	14
Met-Lys-Bradykinin	1318.6968	1318.80 1318.72 1318.70 1318.70 1318.71	1318.73 ± 0.04	25
Bombesin	1618.815	1618.73 1618.90 1618.73 1618.90 1618.72	1618.80 ± 0.10	9

Bioactive Peptide	Theoretical Average Molecular Mass	Calculated Average Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Neuropeptide Y (sheep)	4239.6765	4239.93 4239.68 4239.63 4239.79 4239.63	4239.73 ± 0.13	13

Bioactive Peptide	Theoretical Average Molecular Mass	Calculated Average Molecular Mass	Average ± S.D. (n=5)	Error (ppm)
Neuropeptide Y (porcine)	4253.7034	4253.58 4253.49 4253.78 4253.55 4253.58	4253.60 ± 0.11	24
Neuropeptide Y (human)	4271.7366	4271.93 4271.92 4271.91 4271.94 4271.93	4271.93 ± 0.01	45

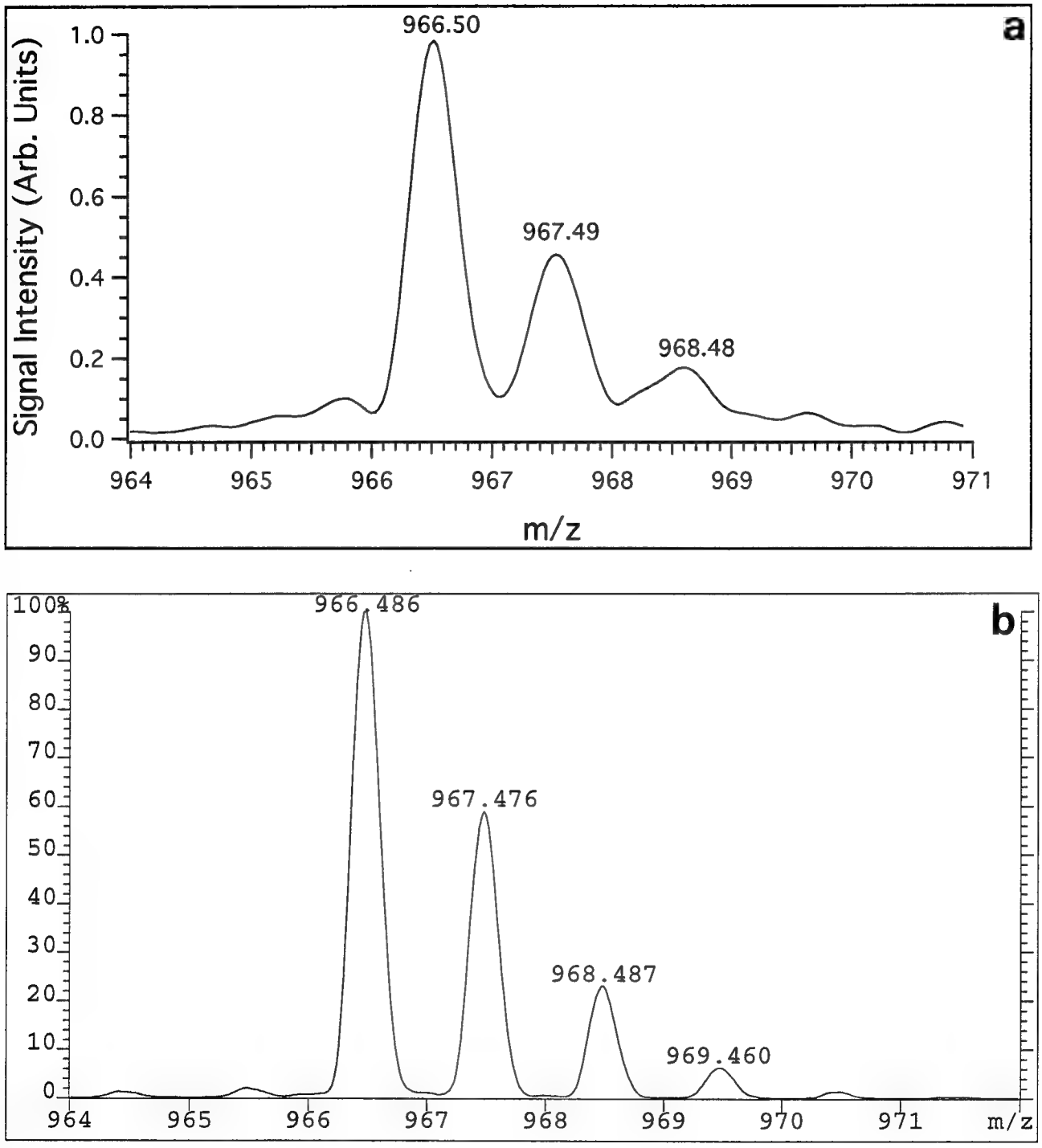

Figure 1: a) MALDI-TOF-MS and b) ESI-MS data acquired during the analysis of substance P (4-11).

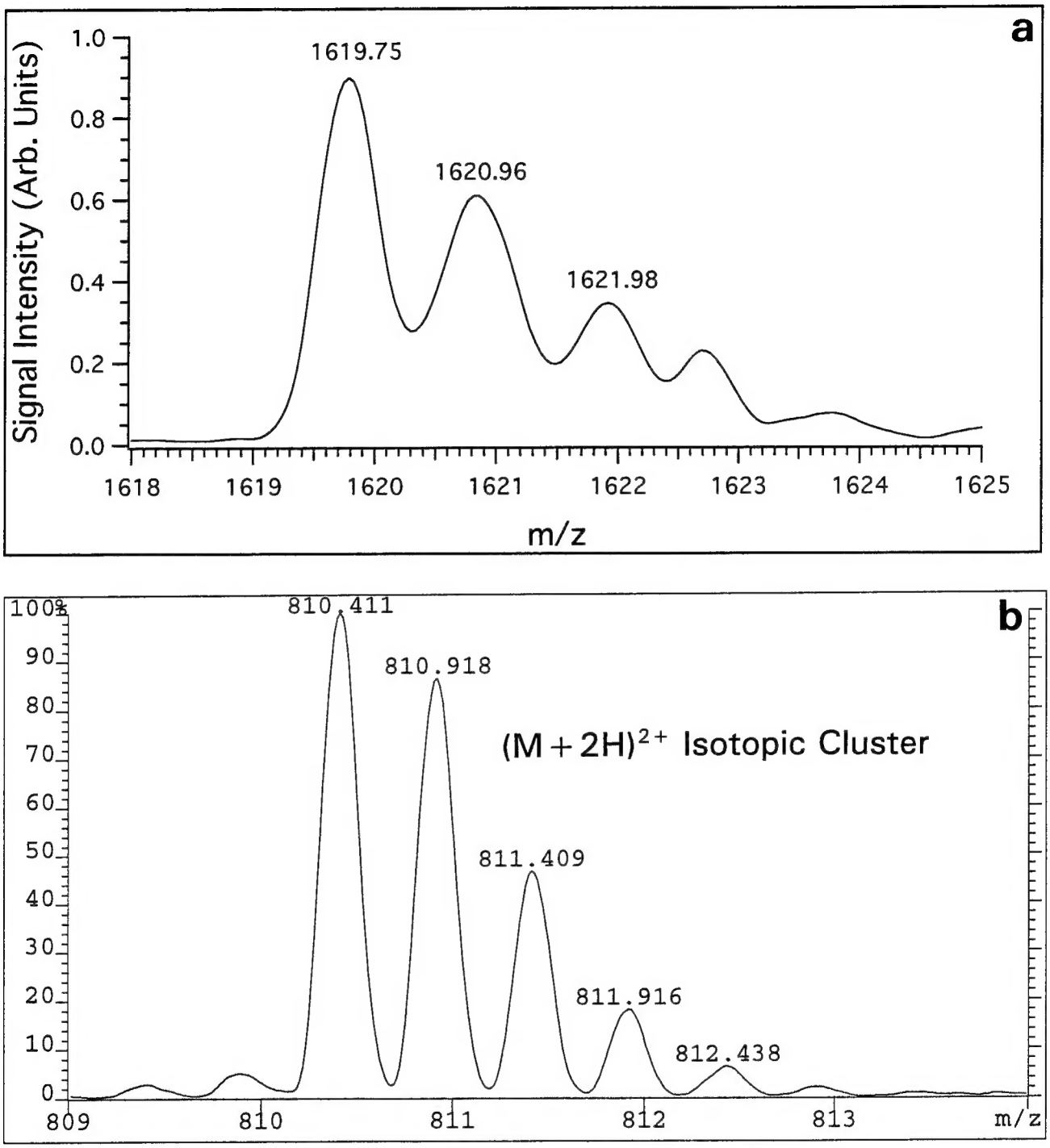

Figure 2: a) MALDI-TOF-MS and b) ESI-MS data acquired during the analysis of bombesin.

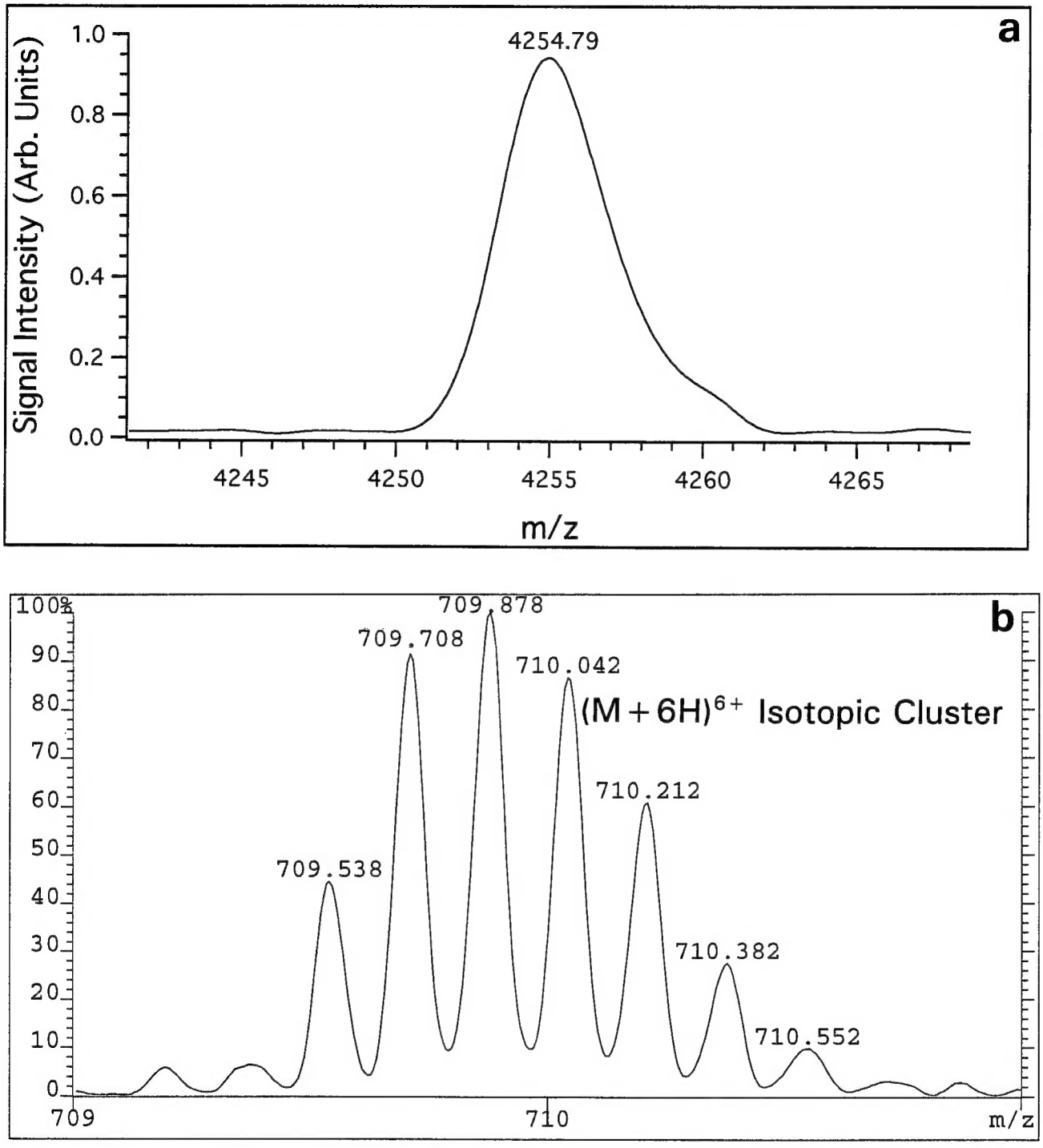

Figure 3: a) MALDI-TOF-MS and b) ESI-MS data acquired during the analysis of neuropeptide Y (porcine).

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Determination of Bioactive Peptide Molecular Mass Using Electrospray and Matrix Assisted Laser Desorption Ionization Mass Spectrometry

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Twelve bioactive peptides, ranging in molecular masses from 600 and 4500 u, were selected for ESI-MS and MALDI-TOF-MS analysis in order to assess the spectrometric data that could be accessed for rapid CB screening purposes. Monoisotopic molecular mass data were obtained for all the peptides by ESI-MS with a tri-focusing magnetic sector instrument in the continuum mode using resolutions between 3000 and 6000 (10% valley definition). MALDI-TOF-MS data were collected with an instrument that featured a four-plate source design, pulsed ion extraction for time-lag focusing, and a 1-m linear flight tube. High resolution mass measurements with the tri-focusing magnetic sector instrument were the most accurate. Errors between theoretical and calculated monoisotopic (or average) molecular mass were in the 0.3 to 18 ppm and 10 to 96 ppm range during ESI-MS and MALDI-TOF-MS analysis, respectively. ESI-MS data may be acquired with as little as 1 to 10 pmoles of peptide at resolutions of 2000 to 3000 (10% valley definition) with the tri-focussing magnetic sector instrument. The MALDI-TOF-MS instrument described offered improved sensitivities with only 0.1 to 1 pmole of peptide being required. In both cases analyses times were rapid, with analyses taking 2 to 5 minutes per sample. Both ionization techniques, ESI and MALDI, would be suitable for rapid molecular mass screening purposes.

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Mass Spectrometry

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Matrix Assisted Laser Desorption Ionization

Bioactive Peptides